REMARKS

Claims 47-49, and 54-66 are pending in the instant application. Claims 1-46 and 50-53 have been cancelled, and Applicants reserve the right to prosecute that subject matter, as well as the originally presented claims, in later applications. Claim 47 has been amended to recite a method for detecting an aptazyme reaction including the step of "providing a substrate comprising a solid support and a heterogeneous mixture of aptazyme constructs covalently immobilized on the solid support." Likewise, new claim 59 recites a method for detecting an aptazyme reaction including the step of "providing a substrate comprising a solid support and an aptazyme construct covalently immobilized on the solid support." Support for these amendments can be found throughout the specification and in the claims as originally filed, and more specifically, support for these amendments can be found at least in claim 47 as originally filed.

New dependent claims 54 and 62 recite methods wherein the "substrate tagged to be detectable is fluorescently tagged, tagged with a magnetic particle, or tagged with an enzyme." Support for these amendments can be found at least at page 15, lines 1-7; at page 60, lines 20-21; and in claims 44, 45 and 46 as originally filed. New dependent claims 55-56 and 63-64 recite methods wherein the solid support is a bead or a well in a multiwell plate. Support for these amendments can be found at least at page 14, lines 12-14; at page 15, lines 1-7; and at page 60, line 22 through page 61, line 2. Dependent claim 57, added herein, is directed to methods wherein "each well contains a bead with an aptazyme construct immobilized thereto which is different from the aptazyme constructs immobilized on the beads located in the other wells of the multiwell plate." Support for this amendment can be found at least at page 14, lines 12-14.

New dependent claims 58 and 65 recite methods wherein the analyte is a metabolite or a protein. Support for these amendments can be found at least at page 14, lines 7-11. New dependent claim 60 is directed to an automated method for detecting an aptazyme reaction. Support for this amendment can be found at least in claim 48 as originally filed, and in the specification at page 11, lines 1-13. New dependent claim 61 recites a method wherein the signal is amplified for detection. Support for this amendment can be found at least in claim 49 as originally filed and in the specification at page 15, lines 10-13

Independent claim 66, added herein, is directed to a method for "detecting an analyte in a sample suspected of containing the analyte by detecting the binding of an aptazyme to a

substrate." Support for this claim can be found throughout the specification and in the claims as originally filed. For example, support for the method of detecting an analyte by detecting the binding of an aptazyme to a substrate can be found at least at page 13, line 19 through page 14, line 1; at page 14, lines 7-22; at page 20, lines 18-21 and at page 60, lines 7-19.

The specification has also been amended herein to correct several typographical errors and to ensure that all trademarked terms are properly identified. Accordingly, no new matter has been added.

Applicants note that the Examiner has acknowledged the Response to Restriction Requirement submitted December 2, 2002. Applicants also note that the priority filing date of June 15, 2000 for United States Application No. 60/212,097 has been acknowledged.

I. Information Disclosure Statement

The Examiner has objected to the listing of references in the specification as an improper information disclosure statement. Applicants filed a Supplemental Information Disclosure Statement on July 31, 2002 in which several of the references listed in the specification were cited. Another Supplemental Information Disclosure Statement will be filed shortly to cite the remaining relevant references listed in the specification.

II. Specification

The Examiner has indicated that "an application in which the benefits of an earlier application are desired must contain specific reference to the prior application(s) in the first sentence of the specification." (Office Action, p. 3). Applicants have amended the specification accordingly and, thus, request that the Examiner withdraw this objection.

The Examiner has also indicated that trademarked terms should be "capitalized wherever they appear and be accompanied by the generic terminology." (Office Action, p. 3). Applicants have amended the specification accordingly and ask that the Examiner withdraw this objection as well.

Applicants also note that the specification has been checked for the presence of possible minor errors. Several typographical and grammatical errors have been corrected herein.

III. Objections to the Claims

Claims 51-53 have been objected to under 37 C.F.R. §1.75 as being substantial duplicates of claim 50. Claims 50-53 have been cancelled herein. Thus, this objection has been rendered moot, and Applicants request that the Examiner withdraw this objection.

IV. Claim Rejections 35 U.S.C. §112, first paragraph

The Examiner has rejected claims 50-53 under 35 U.S.C. §112, first paragraph as containing subject matter that was not described in the specification in such a way as to reasonably convey to one of ordinary skill in the art that the inventors had possession of the claimed invention at the time the application was filed.

Again, Applicants note that claims 50-53 have been cancelled herein. Therefore, any rejection of these claims has been rendered moot, and Applicants request that the Examiner withdraw this rejection.

V. Claim Rejections – 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 47-53 under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. With regard to claim 47, the Examiner has asserted that the phrase "providing a substrate comprising a solid support and an aptazyme construct or a heterogeneous mixture of aptazyme constructs covalently immobilized on the support" is vague and indefinite. According to the Examiner, the sentence structure of claim 47 is confusing. The Examiner has also concluded that the term "the support" in line 5-6 of claim 47 lacks sufficient antecedent basis. With regard to claims 50-53, the Examiner has asserted that the term "the aptazyme" lacks sufficient antecedent basis, while the phrase "modified nucleotides to inhibit degradation of the aptazyme" is vague and indefinite.

Applicants traverse. As noted above, claims 50-53 have been cancelled herein. Thus, any rejection of these claims has been rendered moot and should be withdrawn.

In addition, Applicants have amended the term "the support" in lines 5-6 of claim 47 to recite "the solid support," as suggested by the Examiner. The term "solid support" has sufficient antecedent basis in claim 47 (*see e.g.*, line 3 of claim 47, as amended herein). Accordingly,

Applicants request that the Examiner withdraw this rejection.

Moreover, Applicants contend that the claims, as amended herein, are not indefinite. Claim 47, as originally filed, is directed to substrates that comprise either (i) a solid support and an aptazyme construct covalently immobilized on the solid support, or (ii) a solid support and a heterogeneous mixture of aptazyme constructs covalently immobilized on the solid support. To clarify this, Applicants have amended claim 47 to recite a method for detecting an aptazyme reaction, wherein the method includes the step of providing "a substrate comprising a solid support and a heterogeneous mixture of aptazyme constructs covalently immobilized on the solid support." Applicants have also added a new independent claim, claim 59, which is directed to a method for detecting an aptazyme reaction, wherein the method includes the step of "providing a substrate comprising a solid support and an aptazyme construct covalently immobilized on the solid support." Applicants believe that these claims, as amended herein, are not confusing, vague or indefinite. Applicants, therefore, request that the Examiner withdraw this rejection.

VI. Claim Rejections – 35 U.S.C. § 102

Marshall

The Examiner has rejected claims 47 and 49 under 35 U.S.C. §102(a) as being anticipated by Marshall *et al.*, *Nature Structural Biology*, vol. 6(11):992-94 (1999) ("Marshall"). According to the Examiner, claim 47 is anticipated, because Marshall discloses "aptazyme chips" in which different ribozyme ligases are immobilized on beads in wells to monitor the presence and concentration of different metabolites or proteins. (Office Action, p. 8). The Examiner has also concluded that claim 49 is anticipated, because Marshall discloses the use of "amplification" for increasing the amount of aptamer or aptazyme, thereby amplifying the signal produced. (Office Action, p. 9).

Applicants traverse. Applicants submit herewith a Declaration of Dr. Andrew D. Ellington under 37 C.F.R. §1.131 ("Ellington Decl. I"), which demonstrates that the Marshall reference is unavailable as prior art in the instant application. Dr. Ellington, who is one of the named inventors in the instant application, is also one of the named authors of the Marshall reference. The Marshall reference describes the work of Dr. Ellington. In particular, this publication refers to the work that generated the inventions claimed in the instant application.

(Ellington Decl. I, ¶3-4). Moreover, the inventions claimed in the instant application were invented before the publication date of the Marshall reference. (Ellington Decl. I, ¶5). Thus, the inventions claimed in the instant application were not known or used by others in this country, nor were the methods recited by the instant application described in a printed publication before Applicants invented them. Accordingly, Marshall is not available as prior art under 35 U.S.C. §102(a), and Applicants request that the Examiner withdraw this rejection.

Hesselberth

Claims 47 and 49 have also been rejected under 35 U.S.C. §102(a) as being anticipated by Hesselberth *et al.*, *Reviews in Molecular Biotechnology*, vol. 74:15-25 (2000) ("Hesselberth"). With regard to claim 47, the Examiner has asserted that Hesselberth discloses methods for the "high-throughput construction of chips to sense proteomes and metabolomes." (Office Action, p. 9). In addition, the Examiner has concluded that claim 49 is anticipated by Hesselberth, because this reference discloses ribozymes having appended tags that can be "preferentially amplified." (Office Action, p. 10).

Applicants submit herewith a Declaration of Dr. Andrew D. Ellington under 37 C.F.R. §1.131 ("Ellington Decl. II"), which demonstrates that the Hesselberth reference is unavailable as prior art in the instant application. As described above, Dr. Ellington is one of the named inventors in the instant application and is also one of the named authors of the Hesselberth reference. The Hesselberth reference, which describes the work of Dr. Ellington, refers to the work that produced the inventions claimed in the instant application. (Ellington Decl. II, ¶3-4). Moreover, the inventions recited by the instant application were invented before the publication date of the Hesselberth reference. (Ellington Decl. II, ¶5). Thus, the inventions claimed in the instant application were not known or used by others in this country, and moreover, these inventions were not described in a printed publication before Applicants invented them. Accordingly, Hesselberth is not available as prior art under 35 U.S.C. §102(a), and Applicants request that the Examiner withdraw this rejection.

VII. Claim Rejections – 35 U.S.C. §§102/103

Marshall

Claims 47-49 also stand rejected under 35 U.S.C. § 102(a) "as being anticipated by, or, in the alternative, under 35 U.S.C. §103(a) as obvious over Marshall et al." With regard to claim 48, the Examiner has asserted that, while Marshall does not specifically mention the use of "automation" with the disclosed methods for using "aptazyme chips," automation would have been "immediately envisaged (e.g., anticipated) or in the alternative prima facie obvious" to one of ordinary skill in the art, because chips are "made for automation." (Office Action, pp. 12-13).

Applicants traverse. As described above, the Marshall reference is not available as prior art under 35 U.S.C. §102 or under 35 U.S.C. §103 in the instant application. Accordingly, Applicants request that the Examiner withdraw this rejection.

Hesselberth

Claims 47-49 also stand rejected under 35 U.S.C. § 102(a) "as being anticipated by, or, in the alternative, under 35 U.S.C. §103(a) as obvious over Hesselberth et al." According to the Examiner, Hesselberth does not specifically teach the use of "automation," as recited by claim 48, but automation would have been "immediately envisaged (e.g., anticipated) or in the alternative prima facie obvious" to one of ordinary skill in the art, because chips are "made for automation." (Office Action, p. 14).

Applicants traverse. As described above, the Hesselberth reference is not available as prior art under 35 U.S.C. §102 or under 35 U.S.C. §103 in the instant application. Accordingly, Applicants request that the Examiner withdraw this rejection as well.

VIII. Claim Rejections – 35 U.S.C. §103

Marshall and Cox

Claims 47-49 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Marshall and Cox *et al.*, *Biotechnol. Prog.*, vol. 14:845-850 (1998) ("Cox"). Claims 47 and 49 have been rejected in view of the teachings of Marshall, as discussed above. The Examiner has also rejected claim 48 as obvious in view of the combined teachings of Marshall and Cox. According to the Examiner, Cox teaches that *in vitro* selection can be "automated," and therefore

it would have been obvious to one of ordinary skill in the art to combine the methods of Marshall with the automation processes and equipment disclosed by Cox. (Office Action, p. 16).

Applicants traverse. As described above, the Marshall reference is not available as prior art under 35 U.S.C. §102 or under 35 U.S.C. §103. Applicants also submit herewith the Declaration of Dr. Andrew D. Ellington under 37 C.F.R. §1.131 ("Ellington Decl. III"), which demonstrates that the Cox reference is also unavailable as prior art in the instant application. Dr. Ellington, who is one of the named inventors in the instant application, is also one of the named authors of the Cox reference. The Cox reference describes the work of Dr. Ellington. In particular, this publication refers to the work that produced the inventions claimed in the instant application. (Ellington Decl. III, ¶3-4). Moreover, the inventions recited by the instant application were invented before the publication date of the Cox reference. (Ellington Decl. III, ¶5). Thus, the inventions claimed in the instant application were not known or used by others in this country, and moreover, these inventions were not described in a printed publication before Applicants invented them. Accordingly, Cox is not available as prior art under 35 U.S.C. §102(a) or 35 U.S.C. §103.

As neither Marshall nor Cox is available as a prior art reference in the instant application, Applicants request that the Examiner withdraw this rejection.

Hesselberth and Cox

Claims 47-49 also stand rejected under 35 U.S.C. §103(a) as being unpatentable over Hesselberth and Cox. Claims 47 and 49 have been rejected in view of the teachings of Hesselberth, as discussed above. The Examiner has also rejected claim 48 as obvious in view of the combined teachings of Hesselberth and Cox. According to the Examiner, Cox teaches that *in vitro* selection can be "automated," and therefore it would have been obvious to one of ordinary skill in the art to combine the methods of Hesselberth with the automation processes and equipment disclosed by Cox. (Office Action, p. 16).

Applicants traverse. As described above, neither the Hesselberth reference nor the Cox reference is available as prior art under 35 U.S.C. §102 or under 35 U.S.C. §103 in the instant application. As neither Hesselberth nor Cox is available as a prior art reference in the instant application, Applicants request that the Examiner withdraw this rejection.

Marshall, Cox and Scaringe

Claims 47-53 have also been rejected under 35 U.S.C. § 103(a) as being unpatentable over Marshall and Cox and Scaringe *et al.*, *J. Am. Chem. Soc.*, vol. 120:11820-21 (1998) ("Scaringe"). According to the Examiner, claims 47-49 are rendered obvious by the combined teachings of Marshall and Cox, as described above, and claims 50-53 are obvious in view of the combined teachings of Marshall, Cox and Scaringe.

Applicants traverse. As described above, neither Marshall nor Cox is available as prior art in the instant application. Accordingly, the rejection of claims 47-49 in view of the combined teachings of these references should be withdrawn.

Moreover, claims 50-53 have been cancelled herein. Thus, any rejection of these claims has been rendered moot. Accordingly, Applicants request that the Examiner withdraw this rejection.

Hesselberth, Cox and Scaringe

Claims 47-53 have also been rejected under 35 U.S.C. § 103(a) as being unpatentable over Hesselberth and Cox and Scaringe. According to the Examiner, claims 47-49 are rendered obvious by the combined teachings of Hesselberth and Cox, as described above, and claims 50-53 are obvious in view of the combined teachings of Hesselberth, Cox and Scaringe.

Applicants traverse. As described above, neither Hesselberth nor Cox is available as prior art in the instant application. Therefore, the rejection of claims 47-49 in view of the combined teachings of these references should be withdrawn.

Moreover, claims 50-53 have been cancelled herein. Any rejection of these claims has been rendered moot, and Applicants request that the Examiner withdraw this rejection.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact either of the undersigned at the telephone number provided below.

The Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 23239-301B.

Respectfully submitted,

Dated: May 22, 2003

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Version with Markings to Show Changes Made

In the specification:

The paragraph at page 2, lines 5-8 has been deleted.

The following paragraph has been inserted at page 2, line 1:

--This application claims priority to provisional patent application U.S.S.N. 60/212,097, filed June 15, 2000, which is incorporated herein by reference in its entirety.--

The paragraph at page 2, lines 9-16 has been amended as follows:

--Ribozymes, or RNA enzymes, are oligonucleotides of RNA that can act like enzymes by catalyzing the cleavage of RNA molecules. Generally, ribozymes have the ability to behave like an [endonuleases] endonuclease. The location of the cleavage site is highly sequence specific, approach the sequence specificity of DNA restriction endonucleases. By varying conditions, ribozymes can also act as polymerases or dephosphorylases. –

The paragraph at page 11, lines 14-24 has been amended as follows:

--Automating selection greatly diminishes human error in the actual pipetting and biological manipulations. While programming the robot is often not a trivial task, and can be time consuming, automated selection is far faster and more efficient than manual selection. The scientist's time is thus put to better use preparing samples and analyzing data, rather than performing the actual selection. Additionally, automated selection may include real-time monitoring methods (e.g., molecular beacons, [TaqMan®] TAQMAN®) into the selection procedure and software that can make intelligent decisions based on real-time monitoring.--

The paragraph at page 14, lines 6-11 has been amended as follows:

--It has been shown that ribozyme catalysis can be modulated by allosteric effectors. In yet another embodiment of the present invention, these allosteric ribozymes, also referred to as aptazymes, are [displated] displayed in arrays to be used for monitoring the presence of various molecules, be they inorganic or organic (e.g., metabolites or proteins).--

The paragraph at page 14, lines 12-22 has been amended as follows:

--For example, aptazymes are anchored to a substrate, such as wells in a multi-well plate, and different ribozyme ligases are covalently immobilized on beads in the wells. Mixtures of analytes and fluorescently tagged substrates are added to each well. Where cognate effectors are

present, the aptazymes will covalently attach the [fluoreschent] fluorescent tags to themselves. Where aptazymes have not been activated by effectors, the tagged substrates are washed out of the well. After reaction and washing, the presence and amounts of co-immobilized fluorescent tags are indicative of amounts of ligands that were present during the reaction.--

The paragraph beginning at page 15, line 14 has been amended as follows:

--Nucleic acids are generally less robust than antibodies. However, modified nucleotides may be introduced to the aptazymes that substantially stabilize them from degradation in environments such as sera or urine. Similarly, antibodies generally have higher affinities for analytes than do aptamers, and [be] by inference aptazymes. However, the analytical methods of the present invention do not rely on binding per se, but only on transient interactions. The present invention requires mere recognition rather than actual binding, providing a potential advantage of [apatzymes] aptazymes over antibodies. That is, even low affinities are sufficient for activation and subsequent detection, especially if individual immobilized aptazymes are examined (i.e., by ligand-dependent immobilization of a quantum dot).--

The paragraph beginning on page 23, line 15 has been amended as follows:

--In addition to the containing introns, genomic forms of a gene may also include sequences located on both the 5' and 3' end of the sequences which are present on the RNA transcripts. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the [MRNA] mRNA transcript. The 5' flanking region may contain regulatory sequences such as promoters and enhancers which control or influence the transcription of the gene. The 3' flanking region may contain sequences which direct the termination of transcription, post-transcriptional cleavage and polyadenylation.--

The paragraph beginning on page 32 at line 21 has been amended as follows:

--As used herein, the term "selectable marker" refers to the use of a gene that encodes an enzymatic activity and which confers the ability to grow in medium lacking what would otherwise be an essential nutrient (e.g., the HIS3 gene in yeast cells); in addition, a selectable marker may confer resistance to an antibiotic or drug upon the cell in which selectable marker is expressed. A review of the use of selectable markers in mammalian cell lines is provided in Sambrook, J. [*et. al.*] *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring

Harbor Laboratory Press, New York (1989) pp.16.9-16.15.--

The paragraph beginning on page 35, line 18 has been amended as follows:

As used herein, the term "probe" refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, which is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that nay probe used in the present invention will be labeled with an "reporter molecule," so that it is detectable in any detection system, including, but not limited to enzyme (*e.g.*, [ELISA] ELISA™, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.--

The paragraph at page 36, lines 10-15 has been amended as follows:

--As used herein, the term "target" when used in reference to the polymerase chain reaction, refers to the region of nucleic acid bounded by the primers used for polymerase chain reaction. Thus, the "target" is sought to be sorted [oat] out from other nucleic acid sequences. A "segment" is defined as a region of nucleic acid within the target sequence.--

The paragraph at page 38, lines 1-12 has been amended as follows:

--With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (e.g., hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ^{32}P-labeled deoxynucleotide triphosphates, such as [DCTP] dCTP or [DATP] dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide sequence can be amplified with the appropriate set of primer molecules. In particular the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.--

The paragraph at page 40, lines 12-16 has been amended as follows:

--The following examples illustrate the present invention in the *td* gene system of T4. For a full understanding of the examples, refer to Figures 2a and 2b. The examples are [provide] provided for illustrative purposes and do not limit the scope of the present invention or the scope

of the appended claims.--

The paragraph beginning at page 41, line 14 has been amended as follows:

--A PCR reaction containing 1 µl of the extension dilution, 500 mM KCl, 100 mM Tris-HCl, (pH 9.0), 1% [Triton® x-100] TRITON® X-100, 15 mM $MgCl_2$, 0.4 µM of GpIWt1.75: 5' –GAT AAT ACG ACT CAC TAT AGG GAT CAA CGC TCA GTA GAT GTT TTC TTG GGT TAA TTG AGG CCT GAG TAT AAG GTG-3' (SEQ ID NO:3), 0.4 µM of GpIWt4.89: 5' –CTT AGC TAC AAT ATG AAC TAA CGT AGC ATA TGA CGC AAT ATT AAA CGG TAG CAT TAT GTT CAG ATA AGG TCG TTA ATC TTA CCC CGG AA-3' (SEQ ID NO:4), 0.2 mM each dNTP and 1.5 units of Taq polymerase (Promega, Madison, WI) was thermocycled 20 times under the following regime: 94° C for 30 seconds, 45° C for 30 seconds, 72° C for 1 minute. The PCR reaction was precipitated in the presence of 0.2 M NaCl and 2.5 volumes of ethanol and then quantitated by comparison with a molecular weight standard using agarose gel electrophoresis.--

The paragraph at page 42, lines 5-16 has been amended as follows:

--The aptazyme construct was transcribed in a 10 µl high yield transcription reaction ([AmpliScribe] AMPLISCRIBE™ from Epicentre, Madison, WI). The reaction contained 500 ng PCR product, 3.3 pmoles of P^{32} [[α-32P]] (α-32P)UTP (3000 Camel), 1X [AmpliScribe] AMPLISCRIBE ™ transcription buffer, 10 mM DTT, 7.5 mM each NTP, and 1 µl [AmpliScribe] AMPLISCRIBE ™ T7 polymerase mix. The transcription reaction was incubated at 37° C for 2 hours. One unit of RNase free-DNase was added and the reaction returned to 37° C for 30 minutes. The transcription was then purified on a 6% denaturing polyacrylamide gel to separate the full length RNA from incomplete transcripts and spliced products, eluted and quantitated spectrophotometrically.--

The paragraph at page 43, lines 3-9 has been amended as follows:

--The reactions were terminated by the addition of stop dye (10 µl) (95% formamide, 20 mM EDTA, 0.5% xylene cyanol, and 0.5% bromophenol blue). The reactions were heated to 70° C for 3 minutes and 10 µl was electrophoresed on a 6% denaturing polyacrylamide gel. The gel was dried, exposed to a phosphor screen and analyzed using a Molecular Dynamics [Phosphorimager] PHOSPHORIMAGER™ (Sunnyvale, CA).--

The paragraph at page 45, lines 7-21 has been amended as follows:

--The RNA (10 pmoles/70 µl H_2O) was heated to 94° C for 1 minute then cooled to 37° C over 2 minutes in a thermocycler. The splicing reaction (90 µl) contained 100 mM Tris-HCl (pH 7.45), 500 mM KCl and 15 mM $MgCl_2$. The reaction was immediately placed on ice for 30 minutes. GTP (1 mM) was added to the reaction (final volume of 100 µl) and the reaction was incubated at 37° C for 20 hours. The reaction was terminated by the addition of 20 mM EDTA and precipitated in the presence of 0.2 M NaCl and 2.5 volumes of ethanol. The reaction was resuspended in 10 µl H_2O and 10 µl stop dye and heated to 70° C for 3 minutes and was electrophoresed on a 6% denaturing polyacrylamide gel with [Century™] CENTURY™ Marker ladder (Ambion, Austin, TX). The gel was exposed to a phosphor screen and analyzed. The unreacted RNA was isolated from the gel, precipitated and resuspended in 10 µl H_2O.--

The paragraph at page 46, lines 2-15 has been amended as follows:

The RNA (5 µl of negative selection) was heated to 94° C for 1 minute then cooled to 37° C over 2 minutes in a thermocycler. The positive splicing reaction (45 µl) contained 100 mM Tris-HCl (pH 7.45), 500 mM KCl and 15 mM $MgCl_2$ and 1mM theophylline. The reaction was immediately placed on ice for 30 minutes. GTP (1 mM) was added to the reaction (final volume of 50 µl) and the reaction was incubated at 37° C for 1 hour. The reaction was terminated by the addition of stop dye, heated to 70° C for 3 minutes and was electrophoresed on a 6% denaturing polyacrylamide gel with [Century™] CENTURY™ Marker ladder. The gel was exposed to a phosphor screen and analyzed. The band corresponding to the linear intron was isolated from the gel and precipitated and resuspended in 20 µl H_2O.--

The paragraph at page 46, lines 17-22 has been amended as follows:

The RNA was reverse transcribed in a reaction containing 250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM[,] $MgCl_2$, 0.1 M DTT, 0.4 mM of each dNTP, 2 µM GpIMutG.101 and 400 units of [SuperScript] SUPERSCRIPT™ II reverse transcriptase (Gibco BRL, Rockville, MD). The cDNA was then PCR amplified, transcribed and gel purified as described above.--

The paragraph beginning on page 50, line 22 has been amended as follows:

--Figure 5 is a diagrammatic representation of one embodiment of the exogenous or endogenous activation of Group I intron splicing [is depicted]. A gene of interest 10 is fused to a

reporter gene 12 such as luciferase or beta-galactosidase, which also contains the [group I] Group I intron (td) 14. Splicing-out of the Group I intron is induced by an endogenous effector molecule 16, which may be a protein, e.g., Cyt18. Alternatively, splicing-out of the Group I intron may be induced by an exogenous effector molecule 18. Activation of the aptazyme and auto-excision of the intron results in expression of the reporter gene encoded protein 20 that is [detect] detected by, e.g., fluorescence 22 or any other desired detectable reaction. The use of a reporter gene 12 of this embodiment may be suitable for use in eukaryotic systems.--

In the claims:

Claims 1-46 and 50-53 have been cancelled.

Claim 47 has been amended, and claims 54-66 have been added as follows:

1. - 46. (Cancelled)

47. (Amended) A method for detecting an aptazyme reaction, the method comprising the steps of:

providing a substrate comprising a solid support and [an aptazyme construct or] a heterogeneous mixture of aptazyme constructs covalently immobilized on the solid support;

providing at least one analyte;

providing a substrate tagged to be detectable;

exposing the substrate and at least one analyte to the immobilized aptazymes whereby the substrate is bound to the immobilized aptazymes upon activation of the aptazyme reaction by the analyte to produce a signal;

washing unbound substrate off of the substrate; and

detecting the signal from the bound substrate.

50. - 53. (Cancelled)

54. (New) The method of claim 47, wherein the substrate tagged to be detectable is fluorescently tagged, tagged with a magnetic particle, or tagged with an enzyme.

55. (New) The method of claim 47, wherein the solid support is a bead or a well in a multiwell plate.

56. (New) The method of claim 55, wherein the solid support is a bead in a well of a multiwell plate.

57. (New) The method of claim 56, wherein each well contains a bead with an aptazyme construct immobilized thereto which is different from the aptazyme constructs immobilized on the beads located in the other wells of the multiwell plate.

58. (New) The method of claim 47, wherein the analyte is a metabolite or a protein.

59. (New) A method for detecting an aptazyme reaction, the method comprising the steps of:

providing a substrate comprising a solid support and an aptazyme construct covalently immobilized on the solid support;

providing at least one analyte;

providing a substrate tagged to be detectable;

exposing the substrate and at least one analyte to the immobilized aptazyme whereby the substrate is bound to the immobilized aptazyme upon activation of the aptazyme reaction by the analyte to produce a signal;

washing unbound substrate off of the substrate; and

detecting the signal from the bound substrate.

60. (New) The method of claim 59, wherein the method is automated.

61. (New) The method of claim 59, wherein the signal is amplified for detection.

62. (New) The method of claim 59, wherein the substrate tagged to be detectable is fluorescently tagged, tagged with a magnetic particle, or tagged with an enzyme.

63. (New) The method of claim 59, wherein the solid support is a bead or a well in a multiwell plate.

64. (New) The method of claim 63, wherein the solid support is a bead in a well of a multiwell plate.

65. (New) The method of claim 59, wherein the analyte is a metabolite or a protein.

66. (New) A method for detecting an analyte in a sample suspected of containing said analyte by detecting the binding of an aptazyme to a substrate, the method comprising the steps of:

providing an array having one or more aptazyme constructs disposed thereon at discrete locations by immobilization of said aptazyme constructs on a solid support;

contacting said aptazyme constructs with a substrate tagged with a detectable label, wherein said aptazyme constructs bind to said tagged substrate in the presence of said analyte, but do not bind to said tagged substrate in the absence of said analyte;

contacting said aptazyme constructs and substrate with in a sample suspected of containing said analyte under conditions which allow for substrate binding;

washing away unbound substrate;

detecting the bound substrate, thereby determining the presence of analyte in said sample.